Packing of Large-scale Chromatography Columns with Irregularly Shaped Glass Based Resins Using a Stop-flow Method

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Rigid chromatography resins, such as controlled pore glass based adsorbents, offer the advantage of high permeability and a linear pressure-flow relationship irrespective of column diameter which improves process time and maximizes productivity. However, the rigidity and irregularly shaped nature of these resins often present challenges in achieving consistent and uniform packed beds as formation of bridges between resin particles can hinder bed consolidation. The standard flow-pack method when applied to irregularly shaped particles does not yield well-consolidated packed beds, resulting in formation of a head space and increased band broadening during operation. Vibration packing methods requiring the use of pneumatically driven vibrators are recommended to achieve full packed bed consolidation but limitations in manufacturing facilities and equipment may prevent the implementation of such devices. The stop-flow packing method was developed as an improvement over the flow-pack method to overcome these limitations and to improve bed consolidation without the use of vibrating devices. Transition analysis of large-scale columns packed using the stop-flow method over multiple cycles has shown a two- to three-fold reduction of change in bed integrity values as compared to a flow-packed bed demonstrating an improvement in packed bed stability in terms of the height equivalent to a theoretical plate (HETP) and peak asymmetry (A_s). © 2014 American Institute of Chemical Engineers *Biotechnol. Prog.*, 000:000–000, 2014

Keywords: column packing, stop-flow packing, incompressible resin, ProSep®, controlled pore glass, bed consolidation, large-scale, transition analysis

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Introduction

Chromatography is the workhorse for the purification of biotherapeutics because of its excellent resolving power. In order to meet the stringent purification specifications required by regulatory authorities, it is rare to find a bioprocess design that does not incorporate at least one chromatography step and more often at least two.[1] Such requirements place an increasing demand to design more efficient, reliable, and scalable chromatographic steps. To achieve high product purity, the selection of chromatography resin and process development on the selected resin play a significant role. In addition, the packing efficiency of the chromatography resin can also greatly impact chromatography performance. Packing heterogeneity has long been known to cause band broadening and reduced separation efficiencies.[2,3] A poorly packed column can result in product dilution or insufficient resolving ability to meet the purification objective.[4,5] Regulatory agencies look to biomanufacturers to demonstrate packing consistency and packed bed integrity testing is commonly applied to demonstrate reproducibility of the packing method.[6] Therefore, considerable effort is normally spent in optimizing the column packing method and achieving a homogeneously packed bed can be particularly challenging at large scale due to the complex packing operations required with decrease in aspect ratio (length/diameter) of the column.[7]

Different packing methods and conditions can result in beds that have different structural characteristics.[8–10] Best practices of different packing methods can be found in the respective vendor application notes and industry guidelines.[4] The packing technique applied depends on several critical factors such as the mechanical and physical properties of the resin (including rigidity, particle shape, shear resistance, density, and size) as well as hardware capabilities. For example, soft resins are compressible and cannot tolerate moderate pressures,[6] which will reduce the porosity of the bed or can even disrupt the bed integrity. Therefore, as much as 30% additional resin[6,11] is needed to achieve the required column volume.

Rigid, irregularly shaped adsorbents on the other hand are incompressible under pressure. Bridges are formed between the resin particles[12,13] which may hinder consolidation of the bed during column packing. A packed bed with insufficient consolidation may be unstable and is more prone to further

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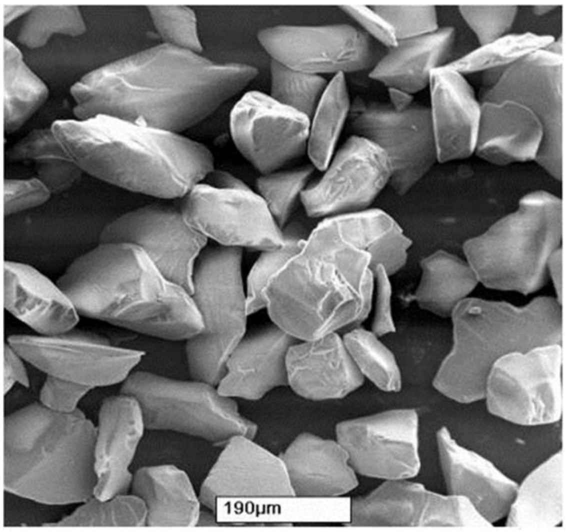

Figure 1. Scanning electron microscopy image of ProSep®-vA HC resin.

With courtesy from EMD Millipore Corporation, MA, USA

consolidate during repeated operation, resulting in a headspace above the settled bed that can have detrimental effects on the separation efficiency and/or result in a target bed height coming out of specification. During commercial manufacturing, this time-dependent effect on the bed characteristics may cause an increasing challenge in satisfying stringent regulatory requirements on product consistency between batches. Repacking of the column may be performed to restore the bed integrity during the manufacturing campaign. However, this effort will spend valuable resources, including time, which will reduce production throughput and increase costs.

Usually some form of mechanical energy is required to disrupt the interparticle bridges in order to achieve adequate bed consolidation during column packing. At laboratory scale, disruption of these bridges can be easily achieved by physically tapping the column. Vibrational energy provided by this tap-packing method has been shown to provide a high level of bed consolidation for packing of rigid resins such as ProSep®-vA High Capacity,[12,14,15] which consists of a base matrix of irregularly shaped, fully rigid, porous glass as shown in Figure 1.

The tap-packing method has been translated to large-scale with the use of a vibration device to mechanically vibrate the column during packing. This approach has been demonstrated to successfully pack chromatography resin in large-scale columns.[14,16,17] Vibration packing methods require the use of pneumatic turbine vibrators clamped to the flange of the column. The number and size of vibrators required increases with increasing column diameter. For large-scale columns of 63 cm diameter or above, 2 to 3 vibrators are needed to supply adequate vibrational energy for the disruption of bridges between the resin particles. The pressure and air flow rate requirements for each vibrator leads to the use of one or more air supply lines. The number of air sources in existing manufacturing suites to supply to process skids and pneumatic vibrators at the same time could be limiting, rendering the application infeasible. Alternatively, conventional flow packing is commonly used but may not provide sufficient bed consolidation.

This work introduces a simple stop-flow method for the packing of rigid, irregularly shaped chromatography resin that is applicable to large-scale columns and does not require the use of additional equipment to provide external mechanical vibration. It has been demonstrated to provide improved bed consolidation compared to conventional flow packing and can be an effective alternative where vibration packing is not feasible.

Experimental Methods

Materials

ProSep®-vA High Capacity (HC) affinity chromatography adsorbent (Merck Millipore, Consett, UK) was used throughout this work. The packing buffer used at pilot and laboratory scale was deionized water. Tris-NaCl buffer was used for large-scale column packing.

Equipment

All studies at laboratory and pilot scales were performed with columns from Merck Millipore, Darmstadt, Germany. Laboratory-scale studies were performed with glass columns of 1.1 cm internal diameter (Vantage® L11x250). Pilot-scale studies were performed with acrylic tube columns of two sizes: 25 cm internal diameter (QuikScale® 250) and 44 cm internal diameter column (IsoPak® 440). The laboratory and pilot-scale columns enabled the process to be observed visually to the packed bed height. Slurry transfer into the IsoPak® 440 column was performed with a slurry transfer skid, STS-1 (Merck Millipore, Darmstadt, Germany). Large-scale column studies were performed with GE Chromaflow® stainless steel tube columns (GE Healthcare, Uppsala, Sweden) with an internal diameter of 140 cm or 180 cm. Slurry transfer was performed using a Chromaflow® packing station (GE Healthcare, Uppsala, Sweden).

Procedure

Slurry Preparation. Storage solution was removed through repeated decanting of supernatant and addition of packing buffer (deionized water for pilot and laboratory scale; Tris-NaCl buffer for large-scale). The target slurry concentration prior to column packing was 50 ± 10% (vol/vol). A target consolidation factor of 1.1–1.15 (refer to "Calculation of Bed Consolidation Factor") was used in preparing the amount of slurry required for packing of the columns.

Packing Methods. *Flow Pack method:* The slurry prepared was transferred into the column. For laboratory and pilot-scale columns, the top adaptor was replaced and reverse (from column bottom to top) flow at 80 cm/h was applied for 20 min to fluidize the slurry to approximately twice the gravity settled bed height. This was followed by forward (from column top to bottom) flow at 1000 cm/h, per resin vendor's recommendationm,[15] for 2 min. For large-scale columns, forward flow at 1,000 cm/h for two column volumes was immediately applied after slurry transfer. The packed bed height was recorded. The top adjuster was subsequently lowered to the packed bed. The packed bed was conditioned with packing buffer in the forward direction at the 1,000 cm/h for one column volume. For the laboratory- and pilot-scale columns, lowering of the top adaptor and conditioning of packed bed was repeated until no further consolidation was observed and the final bed height was recorded.

It was noticed that replacement of the top adaptor for the laboratory and pilot-scale columns after slurry transfer introduced mechanical vibration resulting in extra bed consolidation so the initial reverse flow that was applied was to negate this artifact which may obscure the comparison between the two packing methods. The larger columns that were used did not require removal of the top adaptor during packing and are also less prone to vibration caused by manual manipulation.

Stop-flow Pack Method: The resin slurry was transferred into the column and reverse flow at 80 cm/h was applied for 20 min (for laboratory- and pilot-scale columns) or 30 min for large-scale columns to fluidize the slurry to approximately twice the gravity settled bed height. This was followed by forward flow at 1,000 cm/h for 2 min. At the completion of the initial forward flow, the stop-flow cycle (1 min of no flow followed by 2 min of forward flow) was applied for a total of four times.

For laboratory- and pilot-scale columns, the bed height was recorded after each forward flow that was applied. At the completion of four stop-flow cycles, the top adaptor was lowered to the top of the bed and the bed was conditioned with forward flow at 1,000 cm/h. If further consolidation was observed, the top adaptor was lowered further and conditioning was repeated. The lowering of the top adaptor and conditioning of the packed bed was repeated until no further consolidation was observed, and the final bed height was recorded.

Laboratory-scale Column. The slurry was transferred manually into the column. The top adaptor was then inserted into the column and fittings were connected to the chromatography system. The target bed height for the column was 14 ± 1 cm. An Äktaexplorer™ 100 chromatography system (GE Healthcare, Uppsala, Sweden) was used to supply flow to the column. Before transferring the slurry into the column, the gravity settled bed volume was determined in a measuring cylinder. After transferring the slurry into the column, reverse flow was applied for a minimum of 20 min before the packing method (either flow pack or stop-flow pack) was applied. At the end of packing, the top adaptor was lowered until reaching the top of the packed bed and the final packed bed height was recorded. Packings were performed in duplicates.

Pilot-scale Column. The target bed height for the pilot-scale columns was 20 ± 1 cm. A Quattroflow®-1200 S diaphragm pump (Quattroflow Fluid Systems GMbH & Co KG, Hardegsen-Hevensen, Germany) was used to supply flow to the columns. For packing of the QuikScale® 250 column, slurry was transferred manually (by pouring) into the column. The top adaptor was quickly replaced and lowered into the suspended slurry to prime the top process line. For packing of the IsoPak® 440 column, slurry was transferred into the column using the STS-1 slurry transfer skid. The column top adaptor assembly was set to twice the target bed height and primed before the introduction of slurry from the top slurry valve. The slurry transfer was completed in 5 min. After transferring the slurry into the column, the resin was fluidized and allowed to gravity settle and the bed height after settling was measured.

Reverse flow was applied to the QuikScale® 250 column and the IsoPak® 440 column for a minimum of 20 min in order to re-suspend settled resin in between each pack with the top adaptor raised to twice the gravity settled bed height. The packing methods (either flow pack or stop-flow pack) were carried out on the suspended resin as soon as possible for both columns. At the end of the packing method, the top adaptor was lowered to the top of the packed bed and the final packed bed height was recorded. Packings were performed in duplicate.

Large-scale Column. The 140 cm diameter column was packed to a target bed height of 20 ± 1 cm, whereas the 180 cm diameter column was packed to a target bed height of 14 ± 1 cm. The forward flow applied ranged from 1.1 to 1.2 times the process flow rate. For both columns, the adaptor was set to an initial height of 45 cm for the flow pack method. For the stop-flow pack method, the top adaptor was set to twice the target bed height. The column and transfer lines were then primed with packing buffer. The resin slurry was then transferred into the column from the top slurry valve using a slurry packing skid and the slurry transfer was completed within 5 min. Immediately after transferring the resin slurry, the packing method (either flow pack or stop-flow pack) was applied to the column.

In-process packed bed heights were not recorded for either packing method as the stainless steel columns do not allow any visual observation of packed bed heights. At the end of the packing exercise, the column adaptor was lowered until reaching the top of the packed bed and the final packed bed height was recorded.

Data analysis

Calculation of Bed Consolidation Factor. The bed consolidation factor is defined as the gravity settled bed height or resin volume divided by the packed bed height or volume

$$\text{Consolidation factor}=\frac{\text{Gravity settled bed height or Gravity settled resin volume}}{\text{Packed bed height or Packed bed volume}}$$

The slurry volume employed for the laboratory-scale column was confirmed by gravity settling the required volume of resin in a measuring cylinder. In the above equation, packed bed volumes for the laboratory-scale column was calculated based on measured packed bed heights multiplied by the cross-sectional area. For pilot-scale columns, the consolidation factor was confirmed by gravity settling the resin and measuring the packed bed height directly in the column. For large-scale columns, gravity settled resin volume was determined by employing the PD-10 method[11] on a representative sample collected from the slurry tank. All the resin in the slurry tank was transferred into the large-scale column. Consolidation results were calculated based on the PD-10 result and final packed bed height.

Transition Analysis. Chromaflow® 140 cm and 180 cm diameter columns (GE Healthcare, Uppsala, Sweden) packed with ProSep®-vA HC resin using flow-pack and stop-flow packing methods described in "Packing Methods" and used over repeated cycles during large-scale production of a cell culture product were studied. Chromatography transitions during a buffer wash phase were obtained by exporting production data from a Delta® V Continuous Process Historian (Emerson Process Management, Bloomington, MN) using add-ins compatible with Microsoft® Excel (Microsoft, Redmond, WA). The data consisted of 1-s interval trend data of

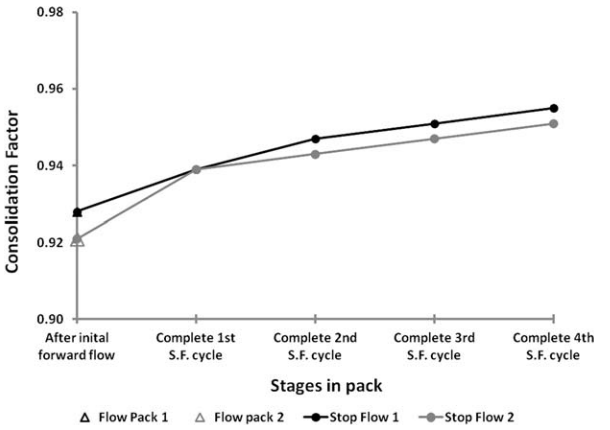

Figure 2. Consolidation of ProSep®-vA HC in QuikScale® 250 mm column during flow packing (black and gray triangle) and stop-flow packing (filled black and gray circle).

Consolidation increased with increasing stop-flow (S.F.) cycles. Minimal improvement in consolidation between 3rd and 4th stop-flow cycle was observed.

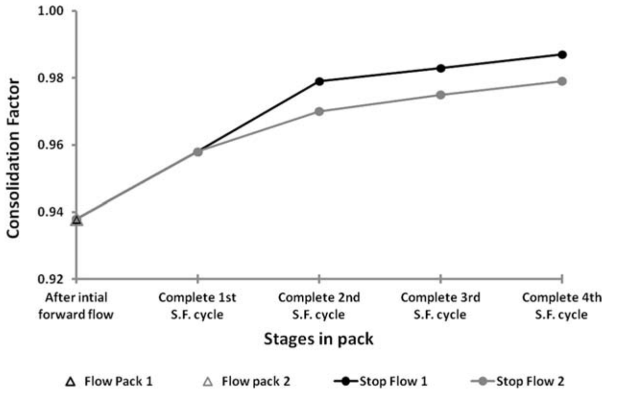

Figure 3. Consolidation of ProSep®-vA HC in IsoPak® 440 mm column during flow packing (black and gray triangle) and stop-flow packing (filled black and gray circle).

Consolidation of packed bed increases with increasing stop-flow (S.F.) cycles. Minimal improvement in consolidation between 3rd and 4th stop-flow cycle was observed.

column exit conductivity paired with time. The data were corrected for baseline offset and normalized to the maximum solute signal during the wash phase. The normalization negated differences in transitions due to buffer makeup or probe standardization.

The method described by Larson et al.[18] was used to calculate non-Gaussian height equivalent to a theoretical plate (HETP) and asymmetry factor (A_s), from the first derivative of the solute signal with respect to time (dC/dt). The derivative dC/dt was estimated numerically using a moving average of the slope, as calculated by linear regression. The non-Gaussian HETP was determined by moment analysis

$$\text{HETP}=\frac{L\sigma^2}{\left(\frac{M_1}{M_0}\right)^2}$$

where L is the column length, σ^2 is the variance, M_1 is the first moment, and M_0 is the zeroth moment.[19] The variance can be expressed in terms of the distribution's moments

$$\sigma^2=\frac{M_2}{M_0}-\left(\frac{M_1}{M_0}\right)^2$$

where M_2 is the second moment.[19]

The asymmetry factor was determined by elution peak analysis

$$A_s=\left(\frac{t_b-t_{\max}}{t_{\max}-t_a}\right)$$

where $t_{\max}$ is the value of time corresponding to $(dC/dt)_{\max}$ and t_b and t_a are the values of time at 10% of $(dC/dt)_{\max}$, such that $t_b > t_a$.[20]

The transition calculations were performed using Microsoft® Excel in combination with Visual Basic (Microsoft, Redmond, WA).

Results and Discussion

Improved packed bed consolidation with stop-flow cycles

Insufficient bed consolidation due to formation of bridges between the resin particles can lead to an out-of-specification packed bed height (higher than expected bed heights). A 25 cm diameter QuikScale® 250 column and a 44 cm diameter IsoPak® 440 column were used to study and optimize the effectiveness of the stop-flow method in improving bed consolidation. The volume of resin was kept the same between the flow-pack and stop-flow packing studies for each column. Figures 2 and 3 show the impact of the number of stop-flow cycles to bed consolidation after the initial forward-flow conditioning. The packed bed was allowed to relax for 1 min ("stop" part of the stop-flow cycle). Subsequently, forward flow at the packing flow rate was applied to the bed ("flow" part of the stop-flow cycle). The packed bed was observed to consolidate further with repeated stop-flow cycles but this becomes insignificant after the third cycle. Hence, four cycles of stop-flow was applied for subsequent studies. A possible explanation of why further consolidation of the packed bed occurs is the relaxation of bridges between the particles during the "stop" part of the cycle, allowing further consolidation to take place with subsequent forward-flow conditioning due to the axial force applied onto the bed which disrupts the bridges to a degree. A high flow, typically 120% maximum process flow rate,[15] is needed to provide sufficient force to disrupt some of the bridging.

The consolidation factor achieved after the 4th stop-flow cycle with the QuikScale® 250 column was 0.95 and 0.98 for the IsoPak® 440 column. The overall improvement in packed bed consolidation using the stop-flow method as compared to the flow-pack method was around 3–4% for both the QuikScale® 250 column and the IsoPak® 440 column. In comparison, packing under the influence of external mechanical energy such as tap-packing and vibration packing method developed by Merck Millipore for ProSep® resins can provide a high consolidation of approximately 1.3 times the gravity settled bed.[14–17]

Impact of column wall effect on packed bed consolidation

Wall effect (a mechanical supporting action produced by the column tube) has been long known to impact the consolidation of chromatographic beds.[21,22] It is a function of the aspect ratio of the packed bed and can support the bed in being more mechanically stable. However, wall support may

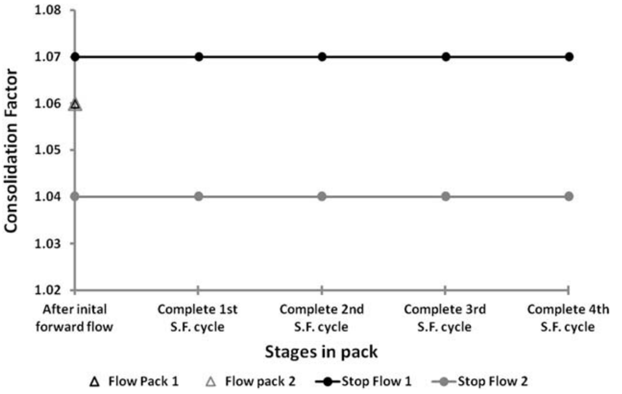

Figure 4. Consolidation of ProSep®-vA HC in Vantage® L11×500 mm column for flow packing (black and gray triangles) and stop-flow packing (filled black and gray circles).

No improvement in consolidation observed during stop-flow (S.F.) cycles. Possible explanation includes wall effect phenomena commonly observed for laboratory-scale columns.

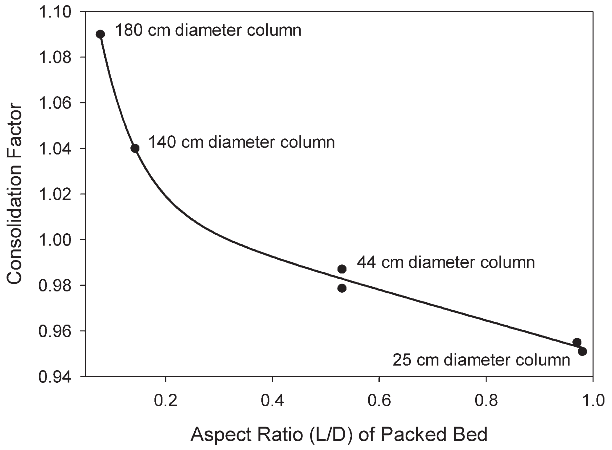

Figure 5. Consolidation factor achieved after stop-flow packing as a function of aspect ratio (length/diameter) of the packed bed.

An increase in consolidation factor as aspect ratio decreases may be due to a reduced wall effect.

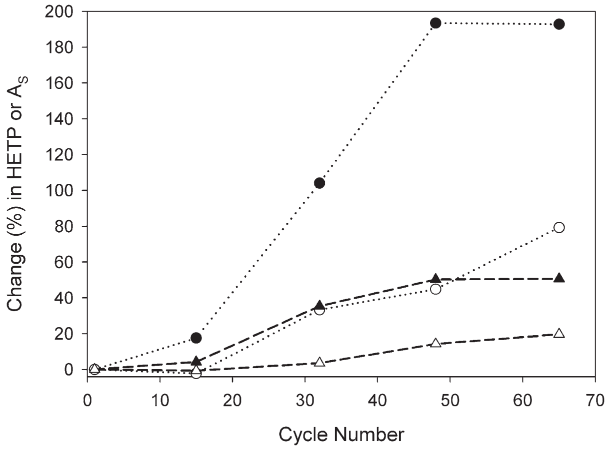

Figure 6. Comparison of change in HETP (circle) and A_s (triangle) for 140 cm diameter columns packed with ProSep®-vA HC resin using flow-pack (filled circle, filled triangle) and stop-flow (un-filled circle, unfilled triangle) packing methods and used over repeated cycles during large-scale production of a cell culture product.

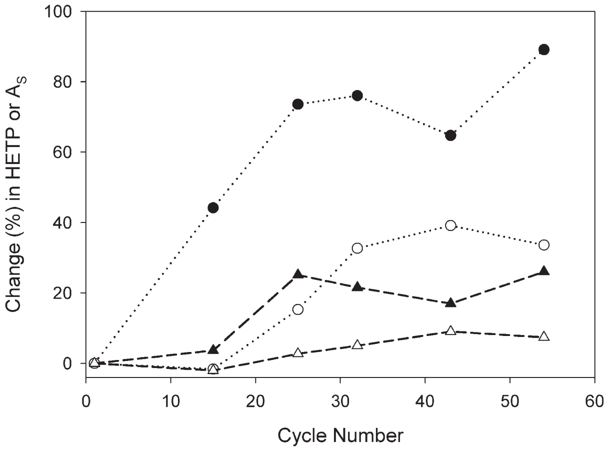

Figure 7. Comparison of change in HETP (circle) and A_s (triangle) for 180 cm diameter columns packed with ProSep®-vA HC resin using flow-pack (filled circle, filled triangle) and stop-flow (un-filled circle, unfilled triangle) packing methods and used over repeated cycles during large-scale production of a cell culture product.

also impede the effectiveness of stop-flow packing as the method relies on the sudden relaxation then further consolidation of the bed with subsequently forward flow to provide mechanical vibration in rearranging the lattice of the resin particles. This impeding phenomenon was observed when the stop-flow method was applied to the laboratory-scale column of 1.1 cm internal diameter. No improvement in bed consolidation was observed with the application of stop-flow cycles (Figure 4).

The effectiveness of the stop-flow packing method appears to increase with a decrease in aspect ratio as the achievable bed consolidation was shown to also increase with a decrease in aspect ratio (Figure 5). This may be due to a decreasing influence of the wall effect, which is caused by the friction of the packed bed against the column wall,[10,21] as the aspect ratio decreases. This friction is responsible for the supporting of the packing structure and can dampen the bed movement during the stop-flow. However, further elucidation of this effect was beyond the scope of this work.

Evaluation of the stop-flow method at large-scale

The stop-flow method that was developed at laboratory and pilot scales was then applied in packing two large-scale columns, namely 140 cm and 180 cm diameter. The consolidation factor of the packed bed obtained after stop-flow packing of ProSep®-vA HC resin in both columns was compared to historically obtained data in the same column after flow packing. The consolidation factor obtained for the 140 cm column increased by a significant 12% (*t*-test, $n = 3$, d.f. = 2, $P < 0.05$) from an average of 0.93 to 1.04 after using the stop-flow method. In fact, the average consolidation factor when using the flow-pack method is less than 1, which suggests uneven settling of particles during the slurry transfer and increased void spaces within the packed bed. The consolidation factor obtained for the 180 cm column increased from an average of 1.04 to 1.09 after using the stop-flow method, which is a significant increase of approximately 5% (*t*-test, $n = 4$, d.f. = 3, $P < 0.05$).

It is important to realize that poor column packing may not only have an immediate impact on bed integrity but can

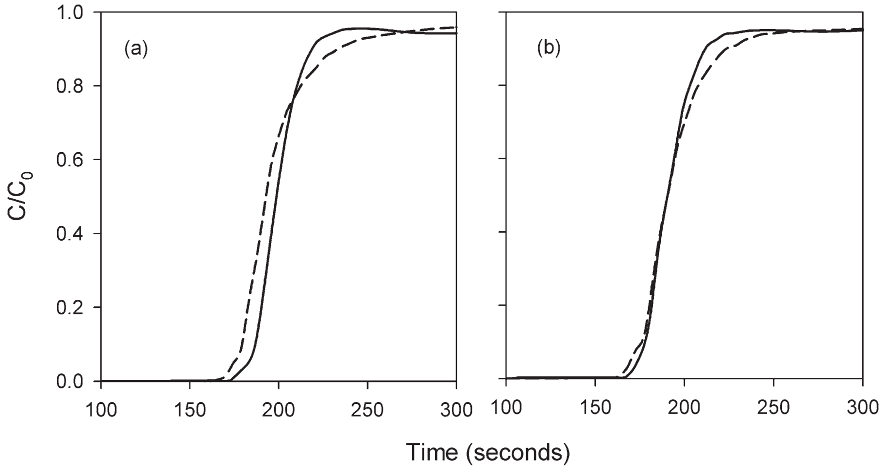

Figure 8. Comparison of transition profiles of normalized conductivity (C/C_0) versus time for 140 cm diameter columns packed with ProSep®-vA HC resin using (a) flow-pack and (b) stop-flow packing methods and used over repeated cycles during large-scale production of a cell culture product.

Profiles for cycle 1 (—, solid line) and cycle 54 (--, dashed line) are shown.

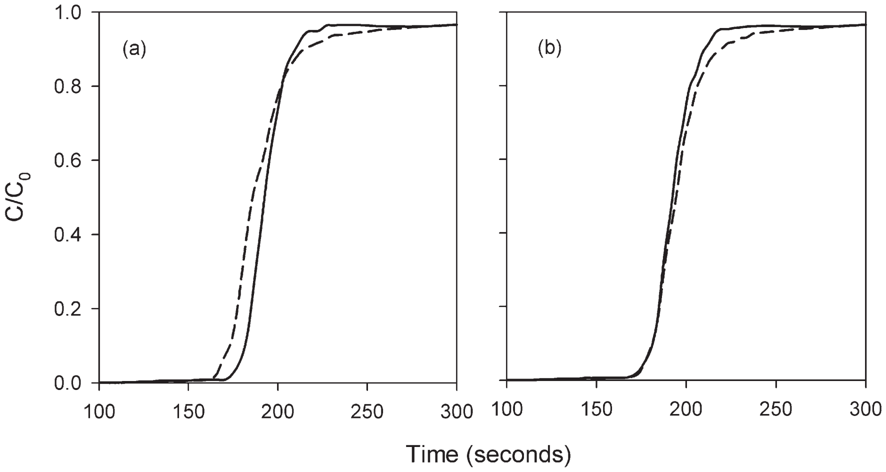

Figure 9. Comparison of transition profiles of normalized conductivity (C/C_0) versus time for 180 cm diameter columns packed with ProSep®-vA HC resin using (a) flow-pack and (b) stop-flow packing methods and used over repeated cycles during large-scale production of a cell culture product.

Profiles for cycle 1 (—, solid line) and cycle 54 (--, dashed line) are shown.

also lead to continuous, time-dependent degradation of bed integrity with repeated use of the column. Transition analysis was used to evaluate the bed stability over repeated use for both 140 cm and 180 cm columns that were packed using the flow-pack method versus the stop-flow method. The initial A_s values obtained after packing for all of the packed beds were greater than 1 (typically between 1.1 and 1.3). The percentage change in HETP and A_s with column cycles for the two packing methods at the two different column sizes are illustrated in Figures 6 and 7. Although deterioration in bed integrity values over repeated cycles was observed for both packing methods, the extent of deterioration for the beds packed with the stop-flow method was substantially lower than that of the corresponding bed that was packed by the flow-pack method. A two- to threefold reduction of change in bed integrity values were observed for stop-flow packed beds as compared to flow-packed beds at the same column size after the last cycle that was analyzed (after approximately 60 cycles) at which the increase in integrity values generally appeared to level off.

The effect of repeated column use on the transition profile is illustrated in Figures 8 and 9 for the 140 cm and 180 cm column, respectively. Stop-flow packed beds at both scales showed a more stable transition profile than the corresponding profiles for the flow-packed beds. For the stop-flow packed beds, broadening occurred mainly in the tailing edge of the profile after multiple cycles; whilst for the flow-packed beds, broadening occurred in both the leading edge and tailing edge. Such broadening may be caused by the formation of a headspace in the column as the bed further consolidates over repeated use. In chromatographic operations, this could lead to loss in column efficiency and product dilution.

A larger increase in HETP and A_s values over repeated cycles was seen for the 140 cm column compared to the 180 cm column where the same packing method was used.

The largest deterioration in bed integrity values over repeated cycles was seen for the 140 cm column packed using the flow-pack method as the initial consolidation factor was on average 0.93, which is less than 1. Generally, the initial bed consolidation achieved after packing for the 140 cm column was considerably worse than that for the 180 cm column, which potentially allowed for more collapsing of the 140 cm bed over time. This may be attributed to the differences in bed height or aspect ratio and hence in the influence of the wall effect as described earlier.

Conclusions

The stop-flow packing method consists of fluidizing the bed followed by a series of starting and stopping forward-flow to the packed bed. This start–stop action disrupts the bridges formed between the resin by allowing the bridges to relax when the flow is stopped and further consolidation to occur when the flow is subsequently resumed.

Development of the method was performed at laboratory and pilot scales using columns with transparent column tubes. It was observed that the increase in bed consolidation (by measuring the bed height) becomes insignificant after three stop-flow cycles. Furthermore, the stop-flow packing method appeared to be more effective with decrease in the aspect ratio of the packed bed. This phenomenon could be due to the decreasing influence of wall effect with decreasing aspect ratio and suggests that this packing method is most effective for packing of large-scale columns.

The stop-flow packing method has been demonstrated to provide significant improvement in bed consolidation during column packing as well as in bed stability over repeated column use, as compared to the flow-pack method. In the studies performed, the stop-flow packing method provided a two- to threefold reduction of change in bed integrity values as compared to the conventional flow-pack method.

Although the extent of bed consolidation as compared to vibrationally packed bed cannot be achieved using this method, the stop-flow packing method can be easily applied without the need of special equipment or extensive utility requirements and proves to be a practical, effective alternative to conventional flow-pack method.

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